E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898

LEGAL DEPARTMENT

June 28, 1985

Mr. Robert Brink
Executive Secretary
Interagency Testing Committee (TS-798)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Dear Mr. Brink:

Hexamethylenediamine (CAS 124-09-4)

This letter provides information supplementing Du Pont's 2/8/85 submission to the ITC for the above captioned chemical. Specifically, this letter clarifies certain statements made in that submission regarding the removal efficiency of Du Pont's Parkersburg, WV site's biopond and provides copies of toxicity studies referenced as ##1, 10 in the 2/8/85 submission at Attachment IV, p. 11.

- Attachment I: Reference #1; Acute Toxicity (Fish) (1965)
- Attachment II: Reference #10(a)-(i)
  - 10(a) Du Pont's files do not currently contain the referenced study.
  - 10(b) Letter report (4/22/47)
  - 10(c) Acute toxicity test (2/3/48)
  - 10(d) Preliminary Report on the toxicity of 1,4-bis(aminocyclohexyl)methane. (HMDA reference at p. 3).
  - 10(e) Evaluation of Acute LC_{50} (bluegill sunfish) (2/19/69)
  - 10(f) Skin primary inhalation test (6/23/69).
  - 10(g) Skin inhalation test on rabbits (6/12/72).
  - 10(h) in vitro microbial mutagenicity studies (7/10/75).
  - 10(i) Oral LD_{50} (rat) (2/23/81).

- Attachment III: The Du Pont literature search erroneously lists the inhalation ten-minute ALC in the mouse as 158 ppm. This number, quoted from the Registry of Toxic Effects of Chemical Substances (RTECS) is incorrect. The original report, upon which the RTECS notation was based, gives the ALC as 7.5 mg/l which is 1575 ppm. Du Pont has provided the ITC a copy of the original reference as Attachment III. The poor quality of the copy is in the original.

With regard to the removal efficiency of the on-site waste water treatment plant (biopond) at Du Pont's Parkersburg site, all process vent streams go through water scrubbers to remove HMDA. The scrubber effluents are directed to an activated sludge wastewater treatment plant. The combined stream from the scrubbers is estimated to contain 200 lbs. per hour of HMDA based on a nonspecific titration test. Treatment system efficiency for specific chemicals is not available, since specific chemical analyses of influent and effluent are not conducted. However, reduction of Biochemical Oxygen Demand (BOD_5) is 99%+. BOD_5 is taken as a measure of the quantity of organic chemicals in a given waste stream. Treatment system influent and effluent BOD_5 are measured four times weekly. HMDA is biodegradable and the BOD_5 factor for it is 1.3 lbs. BOD_5 per lb. of HMDA*. We would expect it to be in the influent stream to the treatment plant at about 400 mg/l and in the effluent stream at less than 5 mg/l.

Very truly yours,

Mark H. Christman

MHC/paa
Atts.

*"Handbook of Environmental Data on Organic Chemicals" by Verschueren, Second Edition.

ATTACHMENT I

DU PONT (HASKELL LABORATORY) LITERATURE REVIEW
REFERENCE #1

ACUTE BIOASSAY STUDIES

for the

E. I. du Pont de Nemours and Company

Academy of Natural Sciences of Philadelphia

Department of Limnology

19th and The Parkway Philadelphia, Penna.

March, 1965

SUMMARY

At the request of Mr. R. L. Hackert of the E. I. du Pont de Nemours and Company, the Limnology Department of the Academy of Natural Sciences of Philadelphia carried out a series of experiments designed to provide information concerning the acute toxicity to fish of two samples labeled, 100 percent Adipic Acid and concentration 76.37% Hexamethylenediamine. These were sent to the Academy by Mr. Hackert of the Seaford Plant of E. I. du Pont de Nemours and Company.

The following results were obtained:

100 percent Adipic Acid

24 and 48 hour TLm 165.7 ppm

B.S.C. 49.7 ppm

Concentration 76.37% Hexamethylenediamine

1. 24 and 48 hour TLm expressed as total sample 0.0105% by volume

B.S.C. expressed as total sample 0.0032% by volume

*2. 24 and 48 hour TLm expressed as Hexamethylenediamine 73.5 ppm

B.S.C. expressed as Hexamethylenediamine 22.4 ppm

*These figures were calculated by the du Pont Company based upon total sample TLm and B.S.C.

INTRODUCTION

At the request of Mr. R. L. Hackert of the E. I. du Pont de Nemours and Company, the Limnology Department of the Academy of Natural Sciences carried out a series of acute bioassay tests on two samples labeled 100 percent Adipic Acid and concentration 76.37% Hexamethylenediamine sent to the Academy by Mr. Hackert. The experimental organism used was the bluegill sunfish, Lepomis macrochirus Raf. The dilution water used was Nanicoke River water sent to the Academy by the Seaford Plant. In all tests a dissolved oxygen concentration of 5 to 9 ppm was maintained. The results, therefore, do not include any deleterious effects that might have been due to an oxygen demand.

This work was carried out by Dr. Arthur Scheier.

METHODS AND PROCEDURES

The Test Organism:

The bluegill sunfish Lepomis macrochirus Raf. was used for test purposes. This fish is found in ponds and lakes and is intermediate in response to many toxic materials.

The fish used in the tests were between 4 and 7 cm. in length, measured from the anterior tip of the head to the end of the caudal fin. In no test did the length of the largest specimen exceed the length of the smallest by more than 50%. The fish were acclimated to the laboratory for a period of one week. They received no food for 36 hours before a test or during a test period, and each fish was used for only one test. Only healthy fish were used. Stock cultures of fish which had more than 5% mortality during the period of acclimatization were discarded.

Apparatus:

The test containers were 5-gallon glass jars. Each was fitted with an aeration tube (7 mm outside diameter) which extended the length of the jar, and a short air vent that also served as an aperture through which a Tygon plastic tubing would be inserted for periodic sampling. The jars were filled with dilution water and placed in a constant temperature vat kept at $22^{\circ} \pm 1^{\circ}C$.

Conditions of the Test:

Dilution water: Natural river water supplied by the Seaford Plant.

Dissolved oxygen 5 to 9 ppm Constant Temperature $22^{\circ} \pm 1^{\circ}C$.

Addition of Test Chemicals:

The volume of sample necessary to produce a given concentration was added to the dilution water and the mixture was oxygenated slowly to prevent foaming.

Addition of Test Organism:

Ten fish, each measuring 4 to 7 cm. in length, were added to the mixed solutions after the dissolved oxygen had been raised to the required leve

Control:

A control jar was maintained with each five test jars. In each control jar 100% survival was required.

Calculation of the Biologically Safe Concentration:

The methods used for calculating the acute biologically safe concentration were those used by Hart et al.[1] and by Doudoroff et al.[2] for testi toxic materials on fresh-water fishes.

These tests were designed to determine the concentration of toxic materials which would kill 50% of the exposed organisms in a given time interval. The concentration of toxic material is expressed as the median tolera limit (TLm). In these tests the chosen time intervals are 24 and 48 hours. The concentration of chemical may be expressed as parts per million (ppm) or per-cent by volume. Thus, for chemical X, a 24 hour TLm equal to 5.2 ppm indicates that a concentration of 5.2 ppm of the chemical would kill 50% of the exposed organisms in 24 hours.

[1]Hart, W. B., P. Doudoroff, and J. Greenbank, 1945. The evaluation of the toxici of industrial wastes, chemicals and other substances to fresh-water fishes. Th Atlantic Refining Company, Philadelphia, Pa. 315 pp.

[2]Doudoroff, P., B. G. Anderson, G. E. Burdick, P. S. Galstoff, W. B. Hart, R. Pa E. R. Strong, F. W. Surber, and W. M. Van Horn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sew. and Ind. Waste 23 (11):1380-1397.

In calculating the biologically safe concentration (B.S.C.) the following equation was used:

$$B.S.C. = \frac{(48 \text{ hr. } TLm)\ (0.3)}{S^2} \quad \text{where } S = \frac{24 \text{ hr. } TLm}{48 \text{ hr. } TLm}$$

Thus the concentration of chemical in the water which would be considered biologically safe is that in which the organisms would be expected to survive.

RESULTS

100 Percent Adipic Acid

Conc. ppm	% Surv. 24 hrs.	% Surv. 48 hrs.
75	100	100
100	100	100
124	100	100
150	100	100
187	0	0
210	0	0

24 and 48 hr. TLm 165.7 ppm
B.S.C. 49.7 ppm

Concentration 76.37% Hexamethylenediamine

Conc. % by Volume of Total Sample	% Surv. 24 hrs.	% Surv. 48 hrs.
0.0065	100	100
0.0087	100	100
0.0135	0	0
0.018	0	0
0.037	0	0

24 and 48 hr. TLm expressed as total sample 0.0105% by volume
B.S.C. expressed as total sample 0.0032% by volume

*24 and 48 hr. TLm expressed as Hexamethylenediamine 73.5 ppm
B.S.C. expressed as Hexamethylenediamine 22.4 ppm

* These figures were calculated by the du Pont Company based upon total sample TLm and B.S.C.

CONCLUSIONS

Both samples tested manifested a major acute lethal effect upon the fish during the first 24-hour period of exposure. If concentrations of these samples approaching the TLm concentration are reached in a stream, the lethal effect will be seen within the first 24-hour period of exposure to these concentrations.

ATTACHMENT II

DU PONT (HASKELL LABORATORY) LITERATURE REVIEW
REFERENCES ##10(a)-(i)

Note: Du Pont has removed from these studies certain information that is clearly not relevant to the assessment of HMDA health and safety (e.g. internal codes; "cc" distribution lists; test results on other chemicals).

REFERENCE 10(a)

Du Pont's files do not currently contain this referenced study.

C
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P
Y

Report 26-47

10(b)

April 22, 1947

HEXAMETHYLENE DIAMINE AND
HEXAMETHYLENE DI-ISOCYANATE

As per our phone conversation of April 21, hexamethylene diamine is a skin irritant and will also irritate the eyes and mucous membrane of the nose and throat. Our experimental work has been on rats only, but concentrations as low as 1% (in vaseline) produced contact irritation of the skin.

We have carried out acute toxicity tests on rats with hexamethylene di-isocyanate and find the oral M.L.D. to be 0.94 grams per kilo body weight. Rats that died showed gastric irritation with edema and cyanosis of the mucosa and some necrosis of the superficial cells of the mucosa. Reports from I.C.I. indicate that hexamethylene di-isocyanate is highly irritant to the skin and eyes. In addition, it is absorbed readily through the skin so that care must be taken to avoid skin contact when the compound is being handled.

HASKELL LABORATORY OF
INDUSTRIAL TOXICOLOGY

John H. Foulger, M.D.
Director

BY: Allen J. Fleming, M.D.
Assistant Director

AJF:dph
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mw

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Report 8-48

10(c)

February 3, 1948

ACUTE TOXICITY TESTS WITH DIISOBUTYL HEXAMETHYLENE DIAMINE, MONOISOBUTYL HEXAMETHYLENE DIAMINE, AND HEXAMETHYLENE DIAMINE (MEDICAL RESEARCH PROJECT

These three compounds have been fed to rats in single large doses and in repeated small doses to determine their acute toxicity. Their effect on the skin has been studied in guinea pigs. The results of the tests are summarized below:

Skin Tests

A. Contact test with the undiluted compounds applied at 1:00 P. M., October 10, 1947:

Time		Effect on the Skin from Contact with: Hexamethylene D	Monoisobutyl HD	Diisobuty
10/20/47	1:10 PM	erythema	erythema	negative
	2:00 PM	skin darkening	skin darkening	VSl eryth
	4:00 PM	Sl necrosis	Sl necrosis	skin dark
10/21/47	8:30 AM	skin necrotic	skin necrotic	skin necr
10/22/47	-------	necrotic areas beginning to separate		

B. Contact and sensitisation test with a 2% aqueous solutio of Hexamethylene Diamine and Monoisobutyl HD and a 2% solution of Diisobutyl HD in absolute alcohol:

	Initial Patch			Final Patch		
	+	Sl+	VSl+	Neg.	+	Sl+	VSl+	Neg.
Hexamethylene D				10				10
Diisobutyl HD		2		8			1	10
Monoisobutyl HD		6	1	3				10

Slight irritation of the skin was noted with Diisobutyl and Monoisobutyl HD in the initial test but not in the final test None of the compounds appear to sensitise guinea pigs. A final

injection given after the customary ten-day rest period did not produce any systemic reaction in the guinea pigs.

Oral Tests

A. Single doses

Compound	Dose in mg/Kilo	Result Following Treatment
Hexamethylene D	100	Survived, gained weight moderately well
	450	Survived, gained weight very slowly
	670	Survived, lost weight
	1000	Died 16 days after treatment (lost 43% of control weight)
	1500	Died overnight
	2250	Died overnight
Monoisobutyl HD	100	Survived, moderate gain in weight
	200	Survived, moderate loss in weight
	300	Survived, marked loss in weight
	450	Died 66 hours after treatment
	670	Died 20 hours after treatment
	1000	Died overnight
Diisobutyl HD	100	Survived, moderate gain in weight
	200	Survived, moderate loss in weight
	300	Survived, moderate loss in weight
	450	Died 23 hours after treatment
	670	Died 18 hours after treatment
	1000	Died 4 hours after treatment

B. Repeated small doses (one fifth of the M.F.D.)

Compound	No. Rats	Dose mg/Kilo	No. Doses	No. Died	Result Following Treat.
Hex.	6	300	10	1 (after 10 T.)	Group failed to gain weight
Mono.	6	90	10	1	Group showed a loss in weight
Di.	6	90	10	0	Group failed to gain weight

Hexamethylene Diamine appears to be somewhat less toxic than the other two compounds, although the general action of all three compounds is much the same and appears to be associated with their caustic properties. This is borne out by the findings at autopsy when the chief pathology noted was in the stomach. The rats fed single doses of the compounds greater than 200 mg/Kilo almost invariably showed an acute hemorrhagic gastritis, and those fed repeated small doses showed slight thickening of the lining of the stomach when sacrificed eleven days after the final

treatment. The loss in weight is attributed to interference with digestion due to the acute gastritis.

The present precautions followed in handling Hexamethylene Diamine should be adequate for handling Monoisobutyl and Diisobutyl Hexamethylene Diamine. Skin contact appears to be the main hazard, and care should be taken to avoid such contact. In case of accidental contact, the skin should be washed at once with plenty of water. In view of the caustic nature of these compounds, safety goggles should be worn when they are being handled. Copious washing of the eye with water should be sufficient if one of these compounds is splashed in the eye accidentally.

HASKELL LABORATORY OF
INDUSTRIAL TOXICOLOGY

John H. Foulger, M. D.
Director

BY: Allan J. Fleming, M. D.
Assistant Director

AJF:lgw
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dyb

10(d)

PRELIMINARY REPORT ON THE

TOXICITY OF 1,4-BIS(AMINOSECBUTYL) BENZENE (PACB)

Comparison with Hexamethylene Diamine

The Approximate Lethal Dose of hexamethylene diamine (HMD) for rats was found to be 1000 mg/kg when the material was administered by stomach tube. The principle effect was a hemorrhagic gastritis with possible injury to the kidneys. The application of undiluted HMD to the skin of guinea pigs resulted in the prompt appearance of erythema and subsequent necrosis. A 2% aqueous solution of HMD produced no irritation or sensitization of guinea pig skin. The vapors of heated hexamethylene diamine (45°C.) are very irritating.

The handling of PACM should require precautions similar to those that have already been taken with HMD. Like HMD, PACM should be treated with the respect due to caustic materials. The fact that PACM is a solid and HMD a liquid should make this task easier. Additional care, however, should be taken to avoid skin contact with PACM, for it is a weak sensitizer.

HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

Report by: Henry Sherman
Henry Sherman
Toxicologist

Approved by: J. Wesley Clayton, Jr.
J. Wesley Clayton, Jr.
Assistant Director

HS/bhh
June 28, 1962
Report No. 51-62

10(c)

WOODARD RESEARCH CORPORATION

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February 19, 1969
Herndon, Virginia

Dr. Gilbert L. Lescanec
Employee Relations Department
Haskell Laboratory
E. I. du Pont de Nemours & Company
Wilmington, Delaware 19898

Dear Doctor Lescanec:

Enclosed are ten copies of our report entitled "Hexamethylene-Diamine (HMDA) - Evaluation of Acute LC_{50} for Bluegill Sunfish."

If you have any questions concerning the report, please let us know.

Sincerely yours,

Carter D. Johnston

Carter D. Johnston, Ph.D.
Biochemist

CDJ:amy

Enclosures

E. I. DU PONT DE NEMOURS AND COMPANY

HEXAMETHYLENE-DIAMINE(HMDA)

EVALUTATION OF ACUTE LC_{50} FOR BLUEGILL SUNFISH

MATERIAL: A bottle containing a light yellow liquid and clear crystals and labeled "Hexamethylene-Diamine, Acute Bioassay Test, HMDA " was received from E. I. du Pont de Nemours and Company, Inc., on August 9, 1968.

A copy of the label is appended.

PROCEDURE: Bluegill sunfish (Lepomis macrochirus), obtained from the George Mazur Enterprises, Washington, D.C., were held for a minimum acclimation period of five days. During this period the fish were fed Purina Trout Chow daily. After acclimation, 100 fish selected to give a population uniform in size were held 72 hours without food in deionized water which was reconstituted by adding $CaSO_4$ (30 mg/l), $MgSO_4$ (30 mg/l), $NaHCO_3$ (48 mg/l), and KCl (3 mg/l). The fish were then placed on experiment as follows:

Material	Concentration ppm	Number of Fish
Control	-	10
Acetone (control)	(1.8 ml)	10
HMDA	56.0	20
HMDA	32.0	20
HMDA	18.0	20
HMDA	10.0	10
HMDA	5.6	10

The experiment was conducted in glass jars, each containing 15 liters of reconstituted water which was aerated prior to the addition of the toxicant and fish. There were five fish per jar. The mean weight of the fish was 2.3 grams and the mean length 5.6 cm (N=10). The water temperature ranged between 66 and 68°F during the course of the experiment.

To each jar was added directly 1.8 ml of an acetone solution of test material such as to achieve the desired concentration. Concentrations were recorded as parts per million (ppm). To each solvent control jar 1.8 ml of acetone was added.

The figures shown are the concentrations immediately after introduction of the test material, and not necessarily the concentrations in the water during or at the end of the test.

RESULTS: The fish were observed frequently during the experiment, and deaths during each 24-hour period were recorded as follows:

Material	Concentration ppm	Cumulative Mortality 24 hrs	48 hrs	72 hrs	96 hrs
Control	-	0/10	0/10	0/10	0/10
Acetone (control)	(1.8 ml)	0/10	0/10	0/10	0/10
HMDA	56.0	0/20	0/20	0/20	0/20
HMDA	32.0	0/20	0/20	0/20	0/20
HMDA	18.0	0/20	0/20	0/20	0/20
HMDA	10.0	0/10	0/10	0/10	0/10
HMDA	5.6	0/10	0/10	0/10	0/10

Duration Hours	HMDA LC_{50}
48	> 56.0 ppm
96	> 56.0 ppm

In addition to the lack of mortality there were no toxic signs observed throughout the course of the experiment.

CONCLUSION: The results of this investigation indicated that HMDA has a 96-hour LC_{50} of >56 ppm in bluegill sunfish. The data that HMDA presents a low toxicity hazard to bluegill sunfish.

Walter B. Knott, B.S.
Biologist

Carter D. Johnston, Ph.D.
Biochemist

Submitted: February 19, 1969

10(f)

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. 164-69

Materials Tested:
Hexamethylenediamine (1,6-hexanediamine)
N,N Diethylhexamethylenediamine (1,6-hexanediamine,N,N'-diethyl)

SKIN PRIMARY IRRITATION TEST

Procedure: To test for primary skin irritation properties, 0.05 ml of each of the two test materials was applied, as received, to the intact shaved shoulder skin of ten male albino guinea pigs.

Results: Within one hour both test materials had produced necrosis over the treated areas of all ten animals. HMD produced a blackish-brown necrotic area in one hour, and one day later this area had a white superficial covering. Skin exposed to NRD-64 became dark red and hemorrhagic within one hour, and after 24 hours was greenish-black with areas of hemorrhagic necrosis. Since NRD-64 spread beyond the site of application, the reactions covered a larger area than those produced by HMD.

Summary: Skin contact with hexamethylenediamine or N,N diethylhexamethylenediamine caused severe necrosis within a very short time. All precautions should be taken to avoid skin contact with either chemical.

Report by: Carol W. Colburn
Carol W. Colburn

CWC:dhg

Approved by:
John A. Zapp, Jr.
Director

Date: June 23, 1969

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. 218-72

Materials Tested: 1) 1,6-Hexanediamine* 6% (w/w) - Aqueous
2) 1,6-Hexanediamine* 10% (w/w) - Aqueous

SKIN IRRITATION TEST ON RABBITS

Part A - 24-Hour Exposure:

Procedure: Six male albino rabbits were clipped free of hair on the trunk and lateral areas and placed in FDA type stocks. 0.5 ml of each of the solutions (6% on the left, 10% on the right) was applied and gently rubbed into the skin with a Teflon® rod. The animals were removed from the stocks after 24 hours and the treated sites were washed with water and dried. Observations were made at 24 hours and seven days.

Results:

6% - 24 hours - One animal negative, the other five reactions included mild to moderate erythema with spots of necrosis.

- 7 days - Two animals negative, four reactions of thick crust formation (necrosis) and desquamation.

10% - 24 hours - Reactions ranged from mild to severe erythema with edema to necrosis. (Six animals)

- 7 days - Reactions included thick crust formation (necrosis) and desquamation. (Six animals)

* Hexamethylene diamine = HMDA

Part B - 30 Second to One Minute Exposure:

Procedure: Six male albino rabbits were clipped free of hair on the trunk and lateral areas. 0.5 ml of each test material (6% on the left, 10% on the right) was applied and gently rubbed into the skin with a Teflon® rod. After a contact period ranging from 30 seconds to one minute, the treated sites were washed with copious amounts of water and dried. Observations were made at 24 and 48 hours.

Results:

6% - 24 hours - Six negative.

- 48 hours - Six negative.

10% - 24 hours - Six negative.

- 48 hours - Six negative.

Summary: 1,6-Hexanediamine, as a 6% or 10% aqueous solution, caused severe skin damage after a 24-hour exposure period. These same materials, when washed from the skin within one minute of application, caused no skin irritation. Extreme care should be used when handling these corrosive materials. It would seem probable that any eye contact with these materials would result in serious eye damage. Splash-proof goggles, protective impermeable gloves and aprons should be worn at all times, and any accidental skin contact should be followed immediately by washing with copious amounts of water.

Report by: Maureen E. McDonnell
Maureen E. McDonnell

Approved by: Charles F. Reinhardt
Charles F. Reinhardt
Assistant Director

MEM:pgh
Date: June 12, 1972

10(h)

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

HASKELL LABORATORY REPORT NO. 378-75

Material Tested: 1,6-Hexanediamine

IN VITRO MICROBIAL MUTAGENICITY STUDIES OF 1,6-HEXANEDIAMINE

Materials and Methods: Three histidine-requiring strains of Salmonella typhimurium were used in the mutagenic assays. Strain TA 1535 is used to detect base-pair substitutions, whereas strains TA 1537 and 1538 are used to detect frame-shift mutations.

The tests were performed in the presence and absence of a rat-liver homogenate metabolic activation system (S-9 mixture). In the absence of metabolic activation, 0.1 ml of a solution of the test compound and approximately 10^8 bacteria were added to 2 ml of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). The solution was mixed and poured on the surface of a Davis minimal agar plate. The metabolic activation system involved the addition of 0.5 ml of S-9 mix to the chemical-top agar solution. The S-9 mix contains per ml: 0.3 ml of the 9000 Xg supernatant of homogenized rat liver, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM sodium phosphate (pH 7.4). This mixture was added directly to the top agar immediately before it was poured over the minimal agar plate.

On the basis of previously determined toxicity data a maximum dose of 100 µg per petri plate was selected for the mutagenic assays.

Appropriate controls were included for each strain. In the non-activated system, these controls consisted of a negative, or solvent control, and a positive control. A second negative control (-S·9 control) is included in the activated assay to measure any activity of the compound in the absence of the S·9 activator mixture.

All plates were incubated for 48 hours at 37°C.

Results: Tables I and II.

Summary: 1,6-Hexanediamine was tested in S. typhimurium tester strains TA 1535, TA 1537 and TA 1538 in concentrations of up to 100 µg per petri plate. The compound was not mutagenic in the microbial assays either in the presence or absence of a liver microsome activation system, i.e., it did not significantly increase the spontaneous or background mutation frequency. The results obtained with and without metabolic activation are presented in Tables I and II, respectively.

Report by: Antje Koops

Antje Koops
Biologist

Approved by: J.G. Aftosmis

J.G. Aftosmis
Manager-Pathology Section

AK:JGA:ljm

Date: July 10, 1975

MUTAGENIC ACTIVITY OF 1,6-HEXANEDIAMINE IN S. TYPHIMURIUM STRAINS TA 1535, TA 1537 AND TA 1538 WITH METABOLIC ACTIVATION

Compound Added	Histidine$^+$ Revertants Per Plate		
	TA 1535	TA 1537	TA 1538
100% Ethanol (Solvent Control)	5	5	14
-S·9 Control*	13	12	5
1,6 HMD µg/Plate			
1 µg/Plate	14	10	19
10 µg/Plate	9	6	17
25 µg/Plate	12	9	19
50 µg/Plate	11	5	18
75 µg/Plate	10	7	16
100 µg/Plate	15	9	22
PC 2AA 10 µg/Plate			~ 3000
100 µg/Plate	227	495	

1,6-HMD = 1,6-Hexanediamine
PC = Positive Control
2AA = 2-Aminoanthracene
* -S·9 Control = Control plate without S·9 activators

TABLE 11

MUTAGENIC ACTIVITY OF 1,6-HEXANEDIAMINE IN S. TYPHIMURIUM STRAINS TA 1535, TA 1537 AND TA 1538 WITHOUT METABOLIC ACTIVATION

Compound Added	Histidine$^+$ Revertants Per Plate		
	TA 1535	TA 1537	TA 1538
100% Ethanol (Solvent Control)	13	14	11
1,6 HMD µg/Plate			
1 µg/Plate	11	9	13
10 µg/Plate	13	8	13
25 µg/Plate	13	8	12
50 µg/Plate	13	14	10
75 µg/Plate	14	3	7
100 µg/Plate	16	12	10
PC MNNG 1 µg/Plate	~ 1100		
9AAc 50 µg/Plate		~ 1100	
2 NF 25 µg/Plate			~ 1000

1,6 HMD = 1,6 Hexanediamine
PC = Positive Control
MNNG = N-Methyl-N'-Nitro-N-Nitrosoguanidine
9AAc = 9-Aminoacridine
2NF = 2-Nitrofluorene

10(i)

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, Newark, Delaware 19711

HASKELL LABORATORY REPORT NO. 111-81

Material Tested
1,6-Hexanediamine*

Study Initiated/Completed
11/4/80-2/11/81

ORAL LD50 TEST IN FASTED AND NONFASTED RATS

Procedure: The test material, as a suspension in corn oil, was administered by intragastric intubation in single doses to 9 groups of 10 nonfasted and 4 groups of fasted young adult Crl:CD® male rats. A Range Finding Study was conducted to determine the initial dose level for the LD50 test.** The surviving rats were weighed and observed during a 14 or 16-day recovery period, and then sacrificed. The LD50 value was calculated from the mortality data using the method of D. J. Finney.***

Results:

FASTED†

Dose (mg/kg)	Average Body Weight (g)	Suspension (%)	Average Dose (ml)	Mortality Ratio	LD50****
1,000	251	6	4.18	8/10	792 mg/kg
800	210	4	4.21	7/10	
700	226	3	5.27	2/10	
500	229	3	3.82	0/10	

NONFASTED

Dose (mg/kg)	Average Body Weight (g)	Suspension (%)	Average Dose (ml)	Mortality Ratio	LD50****
2,000††	247	10	4.95	10/10	1,127 mg/kg
1,600	245	10	3.92	10/10	
1,400	251	8	4.39	9/10	
1,300	258	8	4.19	9/10	
1,250	261	7	4.65	9/10	
1,200	244	7	4.18	0/10	
1,100	254	7	3.98	5/10	
1,000	253	6	4.21	5/10	
800	256	5	4.09	0/10	

Clinical Signs:

FASTED

1,000 mg/kg — Weakness, stained and/or wet perineal area, stained face, moderate weight loss. All deaths occurred with 1 day after dosing.

800 mg/kg — Weakness, lacrimation, stained and/or wet perineal area, stained face, pallor, diarrhea, chromodacryorrhea, and slight to moderate weight loss. All deaths occurred with 2 days after dosing.

700 mg/kg — Weakness, stained and wet perineal area, stained face, diarrhea, congestion, alopecia, and slight weight loss. All deaths occurred with 1 day after dosing.

500 mg/kg — Weakness, stained and wet perineal area, stained face, congestion, and slight weight loss.

NONFASTED

2,000 mg/kg — Salivation. All deaths occurred with 1 day after dosing.

1,600 mg/kg — Weakness. All deaths occurred with 1 day after dosing.

1,400 mg/kg — Weakness, diarrhea, stained and wet perineal area, chromodacryorrhea, stained face, and severe weight loss. All deaths occurred with 2 days after dosing.

1,300 mg/kg — Stained and/or wet perineal area, stained face, diarrhea, weakness, and severe weight loss. All deaths occurred with 2 days after dosing.

1,250 mg/kg	Weakness, stained and/or wet perineal area, stained face, and slight weight loss. All deaths occurred with 1 day after dosing.
1,200 mg/kg	Stained perineal area and slight weight loss.
1,100 mg/kg	Weakness, stained and/or wet perineal area, diarrhea, lacrimation, stained face, and slight to moderate weight loss. All deaths occurred with 2 days after dosing.
1,000 mg/kg	Weakness, stained and/or wet perineal area, stained face, and moderate weight loss. All deaths occurred with 1 day after dosing.
800 mg/kg	Stained and/or wet perineal area, stained face, weakness, congestion, and slight to moderate weight loss.

Summary: 1,6-Hexanediamine is slightly toxic when administered orally to young adult Crl:CD® male fasted and nonfasted rats; its LD50 in fasted rats is 792 mg/kg of body weight and non fasted is 1,127 mg/kg of body weight. Clinical signs most frequently observed were: weakness, stained and/or wet perineal area, stained face, diarrhea, congestion, and slight to moderate weight loss. All deaths occurred within 2 days after dosing.

* Composition: 90.54% 1,6-Hexanediamine
9.46% Water

Synonyms:
- o Hexamethylenediamine
- o 1,6-Diaminohexane

** A Range Finding Study produced deaths at 2,250 mg/kg, after dosing at 670 and 2,250 mg/kg, 1 animal per dose level.

*** Finney, D. J., Probit Analysis, 3rd Ed., 1971, Cambridge University Press.

**** 95% Confidence Limits:

	Fasted Rats	Nonfasted Rats
Lower:	704 mg/kg	594 mg/kg
Upper:	896 mg/kg	1,344 mg/kg
Slope:	11.3	12.6

† Animals were fasted 24 hours prior to dosing.

†† Administered 2 portions, 15 minutes apart.

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ATTACHMENT III

The Du Pont literature search erroneously lists the inhalation ten-minute ALC in the mouse as 158 ppm. This number, quoted from the Registry of Toxic Effects of Chemical Substances (RTECS) is incorrect. The original report, upon which the RTECS notation was based, gives the ALC as 7.5 mg/l which is 1575 ppm. Du Pont has provided the ITC a copy of the original reference as Attachment III. The poor quality of the copy is in the original.

Changes in Status of Compounds Previously Listed. Additional changes in status will be found in Compounds Recommended to be Dropped and Special Studies.

NUMBER	NAME	SOLID or LIQUID		STATUS
	Dibenzarsencie, 5-chloro-	Solid	(At.)	0.97 mg./l. (A), 0/10 mortality (10 days 0.47 mg./l. (N), 0/10 mortality (10 days.
	Dibenzarsenole, 5-cyano-	Solid	(At.)	1.23 mg./l. (A), 1/10 mortality (10 days) 0.65 mg./l. (A), 1/10 mortality (10 days)
	Arsine, dichloro(p-nitrophenyl)-	Solid	(At.)	1.12 mg./l. (A), 20/20 mortality (2 hours)
	4,7-Epoxy-1,3-isobenzofurandione	Solid		N.V. - Man.
	1,6-Hexanediol diisocyanate	Liquid	(At.)	1.33 mg./l. (N), 18/20 mortality 1.07 mg./l. (N), 16/20 mortality 0.94 mg./l. (N), 6/20 mortality 0.56 mg./l. (N), 1/20 mortality
	1,6-Hexanediamine	Solid	(At.)	7.5 mg./l. (FG), 1/20 mortality (1 mg/l = 210 ppm)
	p-Phenylenediamine, N,N,N',N'-tetramethyl-	Solid	(At.)	1.76 mg./l. (FG), 20/20 mortality (2 hours)
	Sesquimustard	Solid	(At.)	1.81 mg./l. (A), 20/20 mortality (4 days)
	Arsine, trioctyl-	Liquid	(At.)	1.58 mg./l. (N), 9/20 mortality (10 days)
	Propane, 1, 2-bis(2-chloroethylmercapto)-	Liquid	(At.)	V - Man. (>12 x LD) 0.69 mg./l. (N), 20/20 mortality (7 days) 0.34 mg./l. (A), 15/20 mortality (10 days) 0.12 mg./l. (N), 5/20 mortality (10 days)
	Arsine, (2-chlorovinyl)diethoxy-			V - Man. (=N-1)